A MORPHOLOGICAL APPROACH OF THE TARSONEMID MITE *STENEOTARSONEMUS SPINKI* SMILEY (TARSONEMIDAE) AS A RICE PLANT PEST*

By

Y. S. Chow, S. S. Tzean, C. S. Chang and C. H. Wang

Institute of Zoology, Academia Sinica, Taipei 115, Taiwan

Synopsis

Chow, Y.S., S.S. Tzean, C.S. Chang and C.H. Wang (Institute of Zoology, Academia Sinica, Taipei 115, Taiwan) : A morphological approach of the tarsonemid mite *Steneotarsonemus spinki* Smiley (Tarsonemidae) as a rice plant pest. *Acta Arachnol.*, **29** : 25-41 (1980).

Results obtained from a study of mouthparts and the digestive system of *Steneotarsonemus spinki* Smiley by using scanning and transmission electron microscopy and physiological staining techniques prove it as an important pest of rice plants. The cheliceral stylet of the mite can puncture the soft surface or epidermis of the rice plant and suck the juices. When the mite population is large in the paddy field, it can also transmit sheath rot disease of rice.

In the second crop season of Taiwan, heavy sterility of rice plants is possibly due to the large spread of this mite. Light and transmission electron micrographs of the mite's digestive system are also shown.

Index descriptors (in addition to those in title) : Ultrastructure, *Acrocylindrium oryzae* Sawada and *Spiroplasm citri*.

I. Introduction

In the southern part of Taiwan, the production of rice plants in the second crop season was lower as compared with the first crop season for the past 5 to 10 years. Part of the reason for the low yielding of rice in the second crop

* This research was supported by the National Science Council NSC-67B-020]-03 (27) Taipei, Taiwan.

season was due to a high rice sterility. When studying the real cause of rice sterility here, we and other entomologists found a large population of the tarsonemid mite *Steneotarsonemus spinki* Smiley, 1967, in a sterile rice plant (Chow *et al.*, 1976; Lo & Hor, 1977). At the same time plant pathologists isolated the rice sheath rot fungus, *Acrocylindrium oryzae* Sawada, from sterile rice plants collected from various localities of Taiwan (Hsieh *et al.*, 1977). Other scientists such as toxicologists, soil chemists, and plant physiologists found that other factors may be involved in rice sterility (Lo & Hor, 1977; Ou *et al.*, 1977). The present paper reports some recent progresses of our laboratory on the study of tarsonemid mites, including what we believe is the first report of the true mechanism of feeding among one of the smallest mites (100 μ-150 μ) of the tarsonemid group.

II. Material and Methods

The tarsonemid mite was collected from a field plot from the Taiwan agricultural experiment station located in the southern part of Taiwan, which was cultivated on the rice variety Tainan 5 from our institute. When the mite reached a very high population density on the host plant, the rice plant was cut with scissors and examined with a Olympus stereo microscope. For scanning electron microscopic observations, the mites on its host plant sheath or alone were mounted on specimen stubs by means of double cellotope and coated with gold in a vacuum evaporator. Micrographs were taken with a JEOL JSE-15 scanning electron microscope at an acceleration voltage of 15 KV. For light and transmission electron electron microscopy, the mites were first fixed in 6% glutaraldehyde in 0.05 M phosphate buffer, pH 7.0 for 2 hr. Following several rinses in the same buffer, the mites were post-fixed in 1% OsO_4 in phosphate buffer adjusted to pH 7.3. They were then washed, dehydrated, embedded in low viscosity resin (Spurr, 1969). Thin sections were cut with glass knife on a Porter Blum MT-2 ultra microtome. For rapid identification, the periodic acid-basic fuchsic fuchsin-methylene blue method was used (Chow *et al.*, 1972). Silver to pale gold sections, after staining with uranyl acetate and lead citrate, were examined in a Hitachi-11-A electron microscope at an accelerating voltage of 50 KV. Micrographs were made with Fuji photographic plates. For physiological absorption experiments of the plant pigment, one group of the mites was inoculated on the leaf sheath of a Taiwan wild rice strain, *Oryzae sativa*

spontanea, which contains a higher quantity of purple pigment, anthocyanin. (MISRO *et al.*, 1960). The other group of mites was cultivated on ordinary rice variety Tainan 5 as control. The mites, after feeding on these 2 different rice plants for 2 days, were examined with a Nikon research microscope and the micrographs taken with Fuji color film and papers. For studying the relationship between the mites and the sheath rot fungus, *Acrocylindrium oryzae* SAWADA, the mites were first collected from a lesion plant and put into a phosphate buffer solution within a centrifugal tube, and centrifuged for 20 minutes at 4000 rpm. The precipitated mites together with spores of the fungus were examined with the scanning or transmission electron microscope as described above.

Results and Discussion

The harvest season for the second rice crop each year in southern Taiwan occurs from August to September. But during this period of time in the paddy field, a large quantity of sterilized rice was observed. Because of the under development of the rice grain, the head of each rice plant did not bend toward the ground due to gravity, but instead grew upright (Fig. 1a). When the plant was examined with a scanning electron microscope, a large number of mites were observed as shown in Fig. 2a. Different stages of the mite under the scanning electron microscope are presented in Fig. 1c to Fig. 3. The mite was first identified as *Steneotarsonemus madecassus* GUTIERREZ by a Taiwanese worker (OU *et al.*, 1977).

However, through the coutesy of Dr. Y. ITO (Japan), the Taiwanese specimens were later sent to Dr. J. GUTIERREZ (New Caledonia) for confirmation of the species name and he concluded that the species from Taiwan should be *S. spinki* SMILEY and apparant by different from *S. madecassus* GUTIERREZ (ITO, 1978, personal communication).

What we were interested in the most was how the mite damages the rice plant. From Fig. 2a, it was not hard to discover that the mite could damage the outside cuticle of the leaf sheath. So the details of the mite's mouthpart was investigated and micrographs were taken of it as shown in Fig. 4. It is very interesting to find that the mouthparts shown here very much resemble those described for *S. pallidus* by HISLOP & JEPPSON (1977). The only difference is that the tip of the cheliceral stylet is further subdivided into 2 pieces (see

Fig. 4c). Because of the sharpness of the stylet, the lesion shown in Fig. 2a was possibly a result of direct damage by the mite. When the population of mites was not very high, the mite was found mostly on the inner surface of the sheath stem. But when the mite population reached very high levels, we could find the mite even in the rice husk (Fig. 2b). By counting the number of mites in a sterilized rice field and those from a normal rice field, we were able to find that the number of mites in the sterilized rice field was higher than that from the control normal field (CHOW *et al.*, 1976). Generally within a population confined to a small area, more female adults are found than males (3 : 1 to 8 : 1). Possibly the mechanism of functional sex ratio also plays a role in this mite (POTTER, 1978). The male always carries and cares for the female deutonymph (Fig. 3b), and this mating behavior has been reporten by COMPTON & KRANTZ (1978) in different mite species. But the question of damage to the rice plant by the mite was still unanswered since there were so many other pests in the rice field, under examination such as leaf hoppers, etc. The lesion observed in Fig. 2a, may still be possibly due to other pests. In order to get more direct evidence, the mite was allowed to feed on a different wild rice strain which contained a higher amount of purple pigment, anthocyanin (MISRO *et al.*, 1960). The results obtained after feeding on this highly pigmented plant was that the digestive system turned purple in color (Fig. 1c and d), in contrast one not fed on this plant, but on the Tainan 5 rice variety. The pigment could also be extracted with ethanol from the plant by a very simple procedure such as shown in Fig. 1b. When comparing the color from the extraction and the mite's digestive tract, there is no doubt that the mite can absorb the juice from its host plant (LEE & CHOW, 1977). From Fig. 1d, we found that the digestive system was very large. So the mite was further studied using standard light and transmission electron microscopical techuiques. For light microscopical studies, the results were not very successful due simply to the fact that the cells of the mite are too small to see any details. Some of the slides are presented in Fig. 5a and b, and only the brain (Br) or synganglionic and caecal (6 m) tissues could be distinguished. These structures were described in detail by WOODRING & COOK (1962), and WOODRING & GALBRAITH (1976), and reviewed by KRANTZ (1971) and EHARA & SHINKAJI (1975) for other related mites. For the transmission electron microscpical studies, the micrographs are presented in Fig. 5c to 6h. In Fig. 5c to Fig. 5e, the brain or synganglion is seen to be penetrated by the esophagus (E). The esophagus is covered with a very thin

but distinct longitudinally pleated cuticular lining that is not round in cross section (Fig. 5e). The caecum (Cm) and other structures are presented in Fig. 6. By comparing the size of the digestive system in Fig. 1d and Fig. 6a, it can be seen that the caecum occupies most of the space in the internal mite cavity. When one sees the enlarged longitudinal or cross section of the caecum (Fig. 6c and d), one finds that the lumen fills with food materials and vacuoles. This is typical of a phytophagous digestive system as described by KUO & NESBITT (1970) and WOODRING & GALBRAITH (1976). Other columnar digestive cells packed with dense granules are also shown in Fig. 6g and h, and it is possible that they are related to a phagocytosis mechanism. These cellular characterizations helped to differentiate it from either the epidermal cell (Fig. 6e and f) or the parenchyma cell (Fig. 6b).

The last part of this investigation involved the study of the relationship between the causal organism of rice sheath rot disease and the mite. When the precipitated mite after centrifugation was observed under the scanning electron microscope, micrograph Fig. 7a was obtained. In this procedure, the density of conidia was increased. Even though conidia were lost during our evaporative preparation, when the specimen was coated in the evaporator, many conidia were still left on the mite's mouthparts and were observed. So the mite can be used as a carrier of the causal organism of the rice sheath rot disease. The result confirmed what has been reported by HSIEH *et al.* (1977) that the tarsonemid mite can transmit the disease. By cutting the mite after centrifugation, no conidia or other mycelial structures were found within the mite, but many conidi were found on only the surface of mite cuticle, as shown in Fig. 7b, and d. Besides *A. oryzae*, a mycroplasm like organism *Spiroplasm citri* was also found associated with the mite for the first time. (Fig. 7c). Recently, Dr. C. N. CHEN from the Taiwan Plant Protection Center (personal communication) found a very high correlation coefficient between the mite sterility of rice plant by using biometrical analysis. All the above results give good evidence that the mite is a very important rice plant pest in Taiwan.

Acknowledgements

We thank Father Dr. Franz Huber, Chairman of the Biology Department, Fu-Jen University, Taiwan, for the permission of using the scanning electron microscope and to Dr. Franklin Chang, Department of Entomology, University of Hawaii, U. S. A. for his review of the manuscript, and the correction of the English.

Literature Cited

CHOW, Y.S., S.H. LIN & J.S. SU., 1972. A new tarsal gland of the brown dog tick, *Rhipicephalus sanguineus* (Latreille), 1804 (Acarina: Ixodidae). *Bull. Inst. Zoo., Academia Sinica,* **11** : 35–39.

CHOW, Y.S., T.L. LEE, H.P. WU, K.C. HSIEH & C.H. WANG, 1976. Survey of the mite pest in a sterile rice field. *Nat. Scien. Coun. Mon. Taiwan.,* **4** : 38–43. (in Chinese).

COMPTON G.L. & G.W. KRANTZ, 1978. Mating behavior and related morphological specialization in the Uropodin mite, *Caminella peraphora*. *Science,* **200** : 1300–1301.

EHARA, S. & N. SHINKAJ, 1975. An Introduction to Agricultural Acarology. 328 pp. Henkoku Noson Kyoiku Kyokai, Tokyo, Japan. (in Japanese).

HISLOP, R.G. & L.R. JEPPSON, 1976. Morphology of the mouthparts of several species of phytophagous mites. *Ann. Entomol. So. Amer.,* **69** : 1125–1135.

HSIEH, S.P.Y., W.L. LIANG & S.Y. CHANG, 1977. Etiological studies on the sterility of rice plant I. Association of sheath rot fungus, *Acrocylindrium oryzae* Sawada, with sterile rice plant. *Plant Prot. Bull. Taiwan,* **19** : 30–36. (in Chinese).

KRANTZ, G.W., 1971. A Manual of Acarology. 335 pp. O.S.U. Book Stores, Inc. Corvallis, Oregon U.S.A.

KUO, J.S. & H.H. NESBITT, 1970. The internal morphology and histology of adult *Caloglyphus mycophagus* (Megnin). (Acarina: Acaridae). *Can. J. Zool.,* **48** : 505–510.

LEE, T.L. & Y.S. CHOW, 1977. Direct evidences for the damage of rice plant by tarsonemid mite, *Steneotarsonemus madecassus* (Acarina: Tarsonemidae). *Nat. Scien. Coun. Mon. Taiwan,* **5** : 960–963. (in Chinese).

LO, K.C. & C.C. HOR, 1977. Preliminary studies on rice tarsonemid mite, *Steneotarsonemus spinki* (Acarina: Tarsonemidae). *Nat. Scien. Coun. Mon. Taiwan,* **5**(4) : 274–284.

MISRO, B.R. SEETHARAMAN & R.H. RICHHARIA, 1960. Studies on world genetic stock of rice: I. Patterns of anthocyanin pigment distribution. *Ind. J. Genet. Plant Breed.,* **20** : 113–117.

OU, Y.T., H.C. FANG & Y.H. TSENG, 1977. Studies on *Steneotarsonemus madecassus* Gutierrez of rice. *Plant Prot. Bull. Taiwan,* **19** : 21–29.

POTTER, D.A., 1978. Functional sex ratio in the carmine spider mite. *Ann. Entomol. Soc. Amer.,* **71** : 218–222.

SPURR, A.R., 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.,* **26** : 31–43.

WOORRING, J.P. & E.F. COOK, 1962. The internal anatomy, reproductive physiology, and molting process of *Ceratozetes cisalpinus* (Acarina: Oribatei). *Ann. Entomol. Soc. Amer.,* **55** : 164–181.

WOODRING, J.P. & C.A. GALBRAITH, 1976. The anatomy of the adult uropodid *Fuscouropoda agitans* (Arachnida: Acari), with comparative observations on other acari. *J. Morph.,* **150** : 19–58.

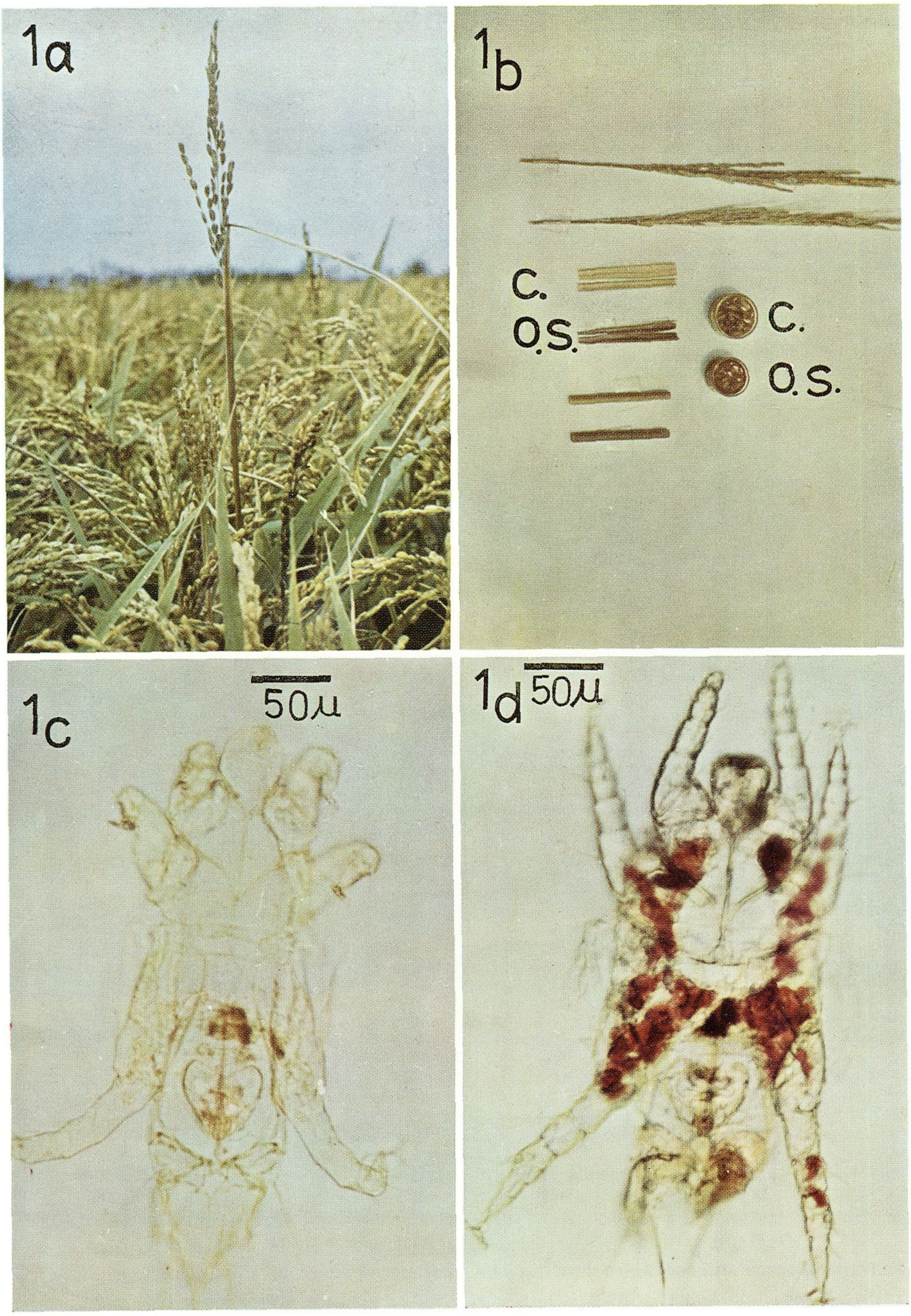

Fig. 1. Direct evidence of the absorption of plant juice by the tarsonemid mite *Steneotarsonemus spinki*.—a. A typical sign of sterility in rice grains in Taiwan.—b. Direct extraction of the purple color pigment (anthocyanin) from the experimental rice plant *Oryzae sativa spontanea* (O. S.) and control rice variety Tainan 5 (C). The anthocyanin was extracted with ethanol in a small container. c. Male mite after feeding on the rice variety Tainan 5. Its digestive system is seen not to stain. d. Male mite after feeding on the rice plant *Oryzae sativa spontanea*. Its digestive system shows an intensive purple to red color.

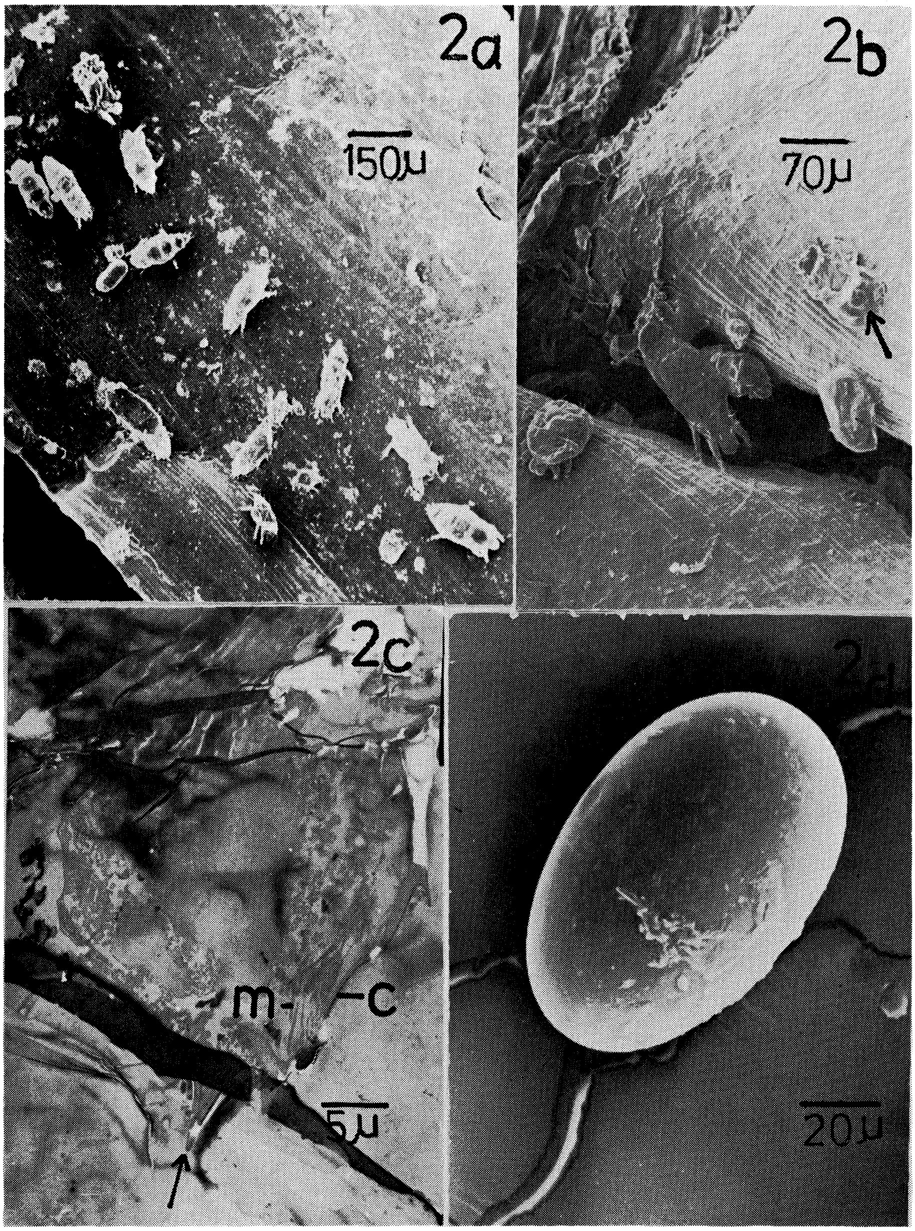

Fig. 2. Damage caused by the mite on the internal surface of a rice plant. a. A scanning electron micrograph showing the surface damage of the leaf sheath. b. The mite with its mouth parts inserted into the inner surface tissue of a rice husk. Note that its legs are not directly attached to its host plant (arrow). c. A transission micrograph showing a cross-section of the mouth parts and the head of the mite. C : cuticle. M : muscle. Arrow : possible chelicerae stylets. d. An enlarged scanning electron microscopic view of a mite egg from (a).

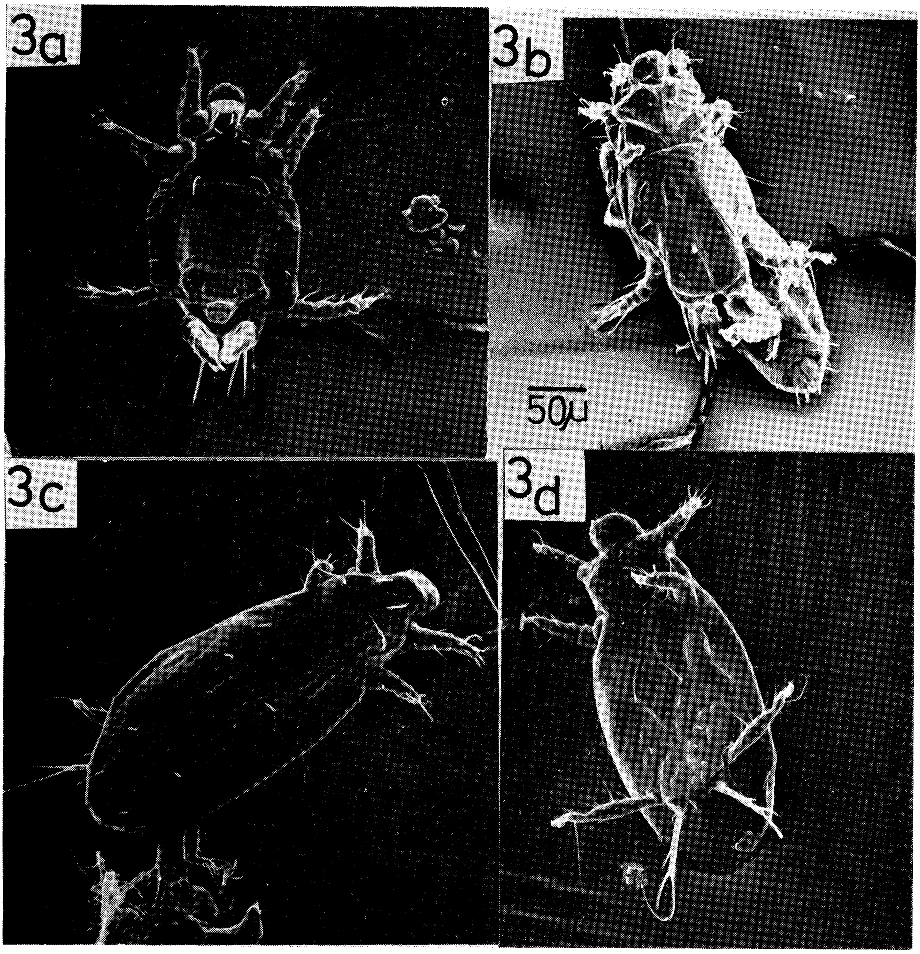

Fig. 3. Enlarged scanning electron micrographs of the mites. a. Dorsal view of the male adult. Note an anal sucker at the posterior. b. Ventral view of the male and the nymph underneath. c. Dorsal view of the female. d. Ventral view of the female.

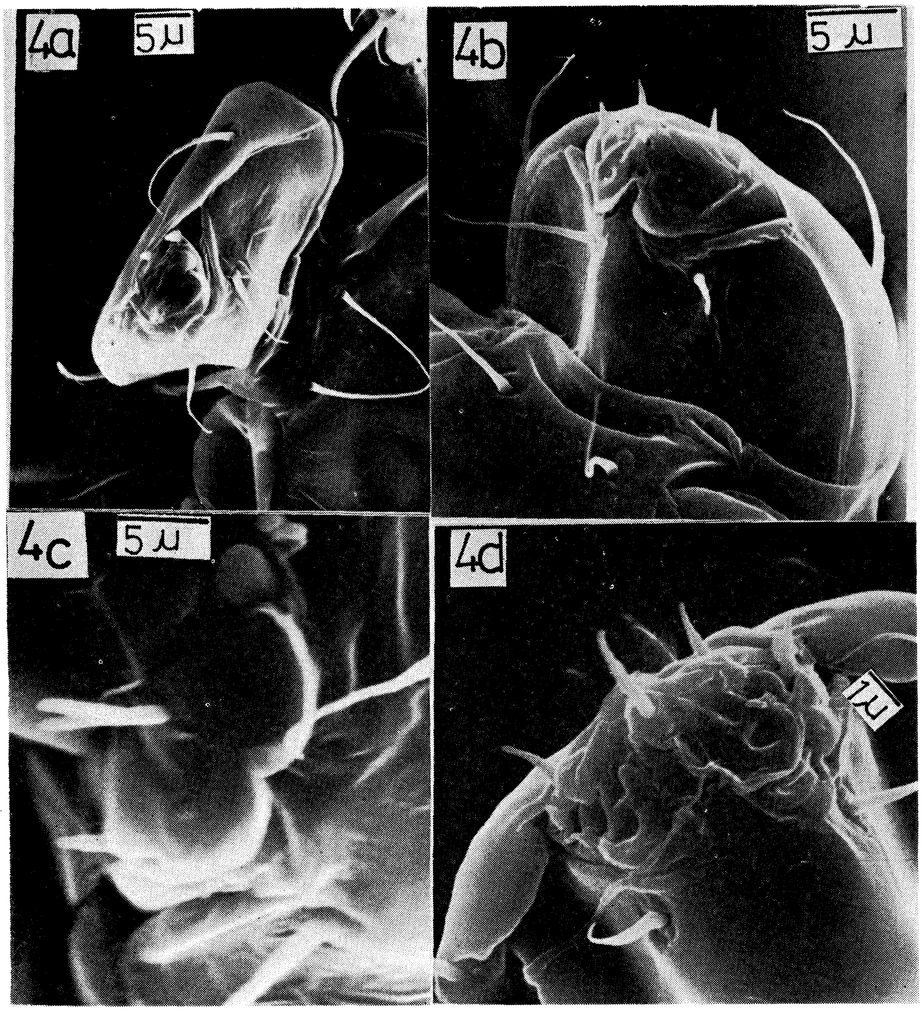

Fig. 4. Enlarged scanning electron micrographs of the female mouthparts. a. The head with cheliceral seta exposed and cheliceral stylets within the lips. b. Same as (a) but cheliceral stylet half extended. c. Another specimen showing that the cheliceral stylet can be further subdivided into 2 parts at the tip. d. Full extended cheliceral stylet of the female mite.

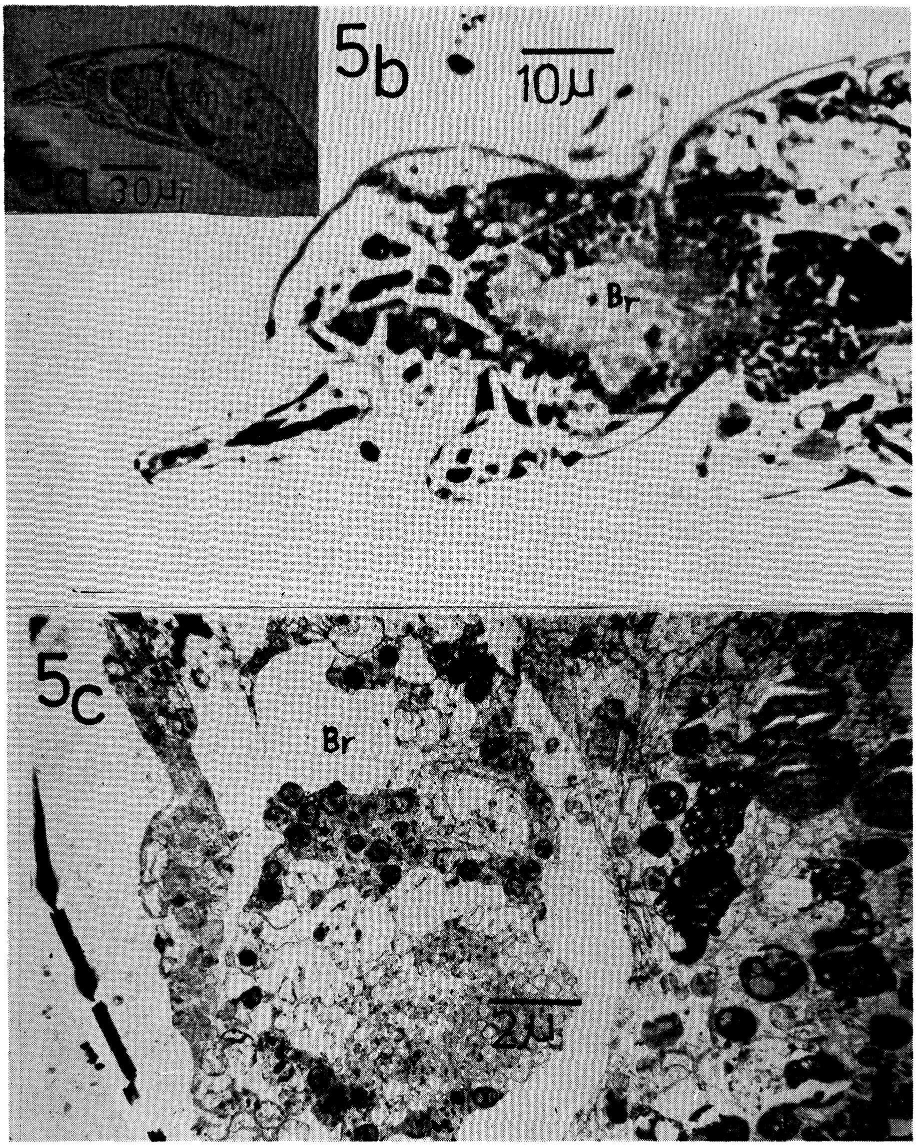

Fig. 5. Nervous system of the mite. a. Longitudinal section of the mite showing the brain (Br) and caecum (Cm) under light microscopy. Periodic acid-basic fuchsin methylene blue stain. b. Some as (a) but enlarged view of the brain (Br). c. Electron microscopical view of the brain (Br) mass.

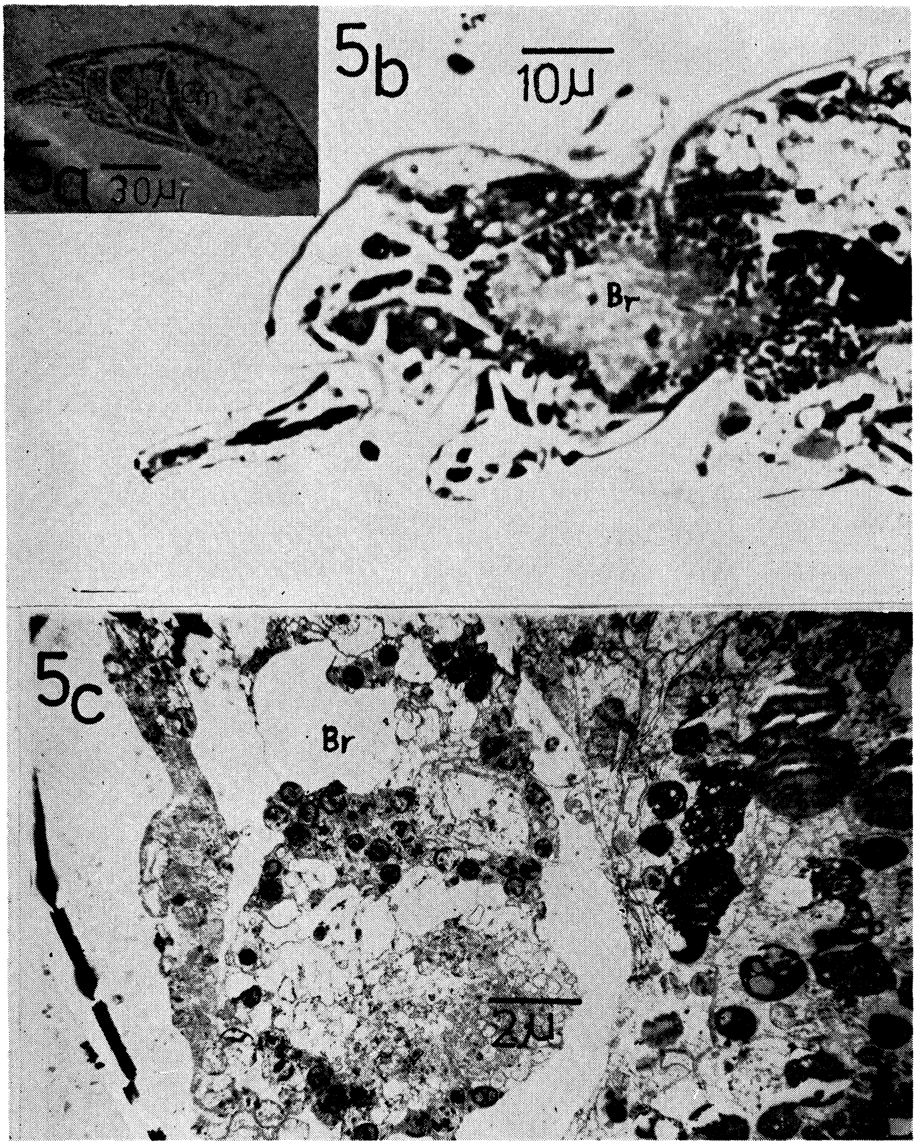

Fig. 5. Nervous system of the mite. a. Longitudinal section of the mite showing the brain (Br) and caecum (Cm) under light microscopy. Periodic acid-basic fuchsin methylene blue stain. b. Some as (a) but enlarged view of the brain (Br). c. Electron microscopical view of the brain (Br) mass.

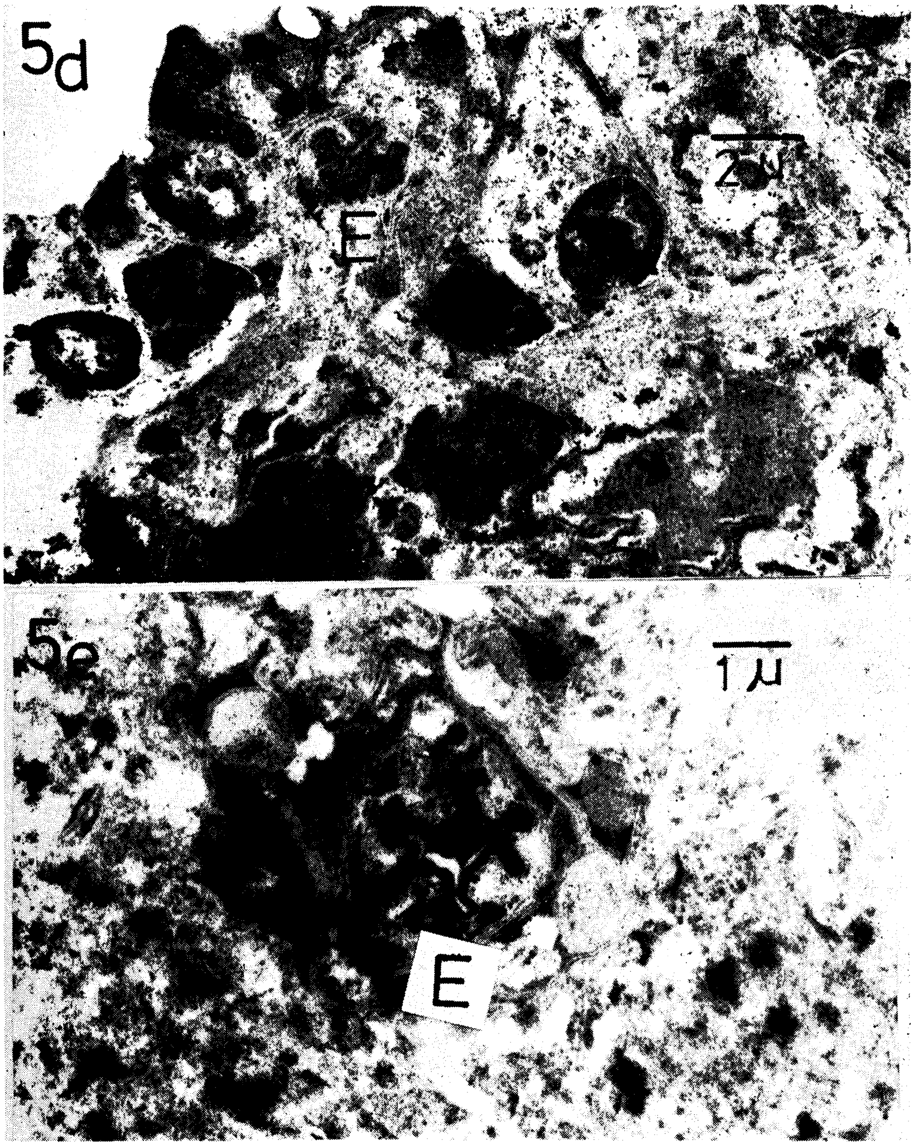

Fig. 5. d. Cross section of the brain showing the esophagus (E) passing through the cortex region of the brain. e. Same as d, but the esophagus (E) passing through the center or white mass of the brain.

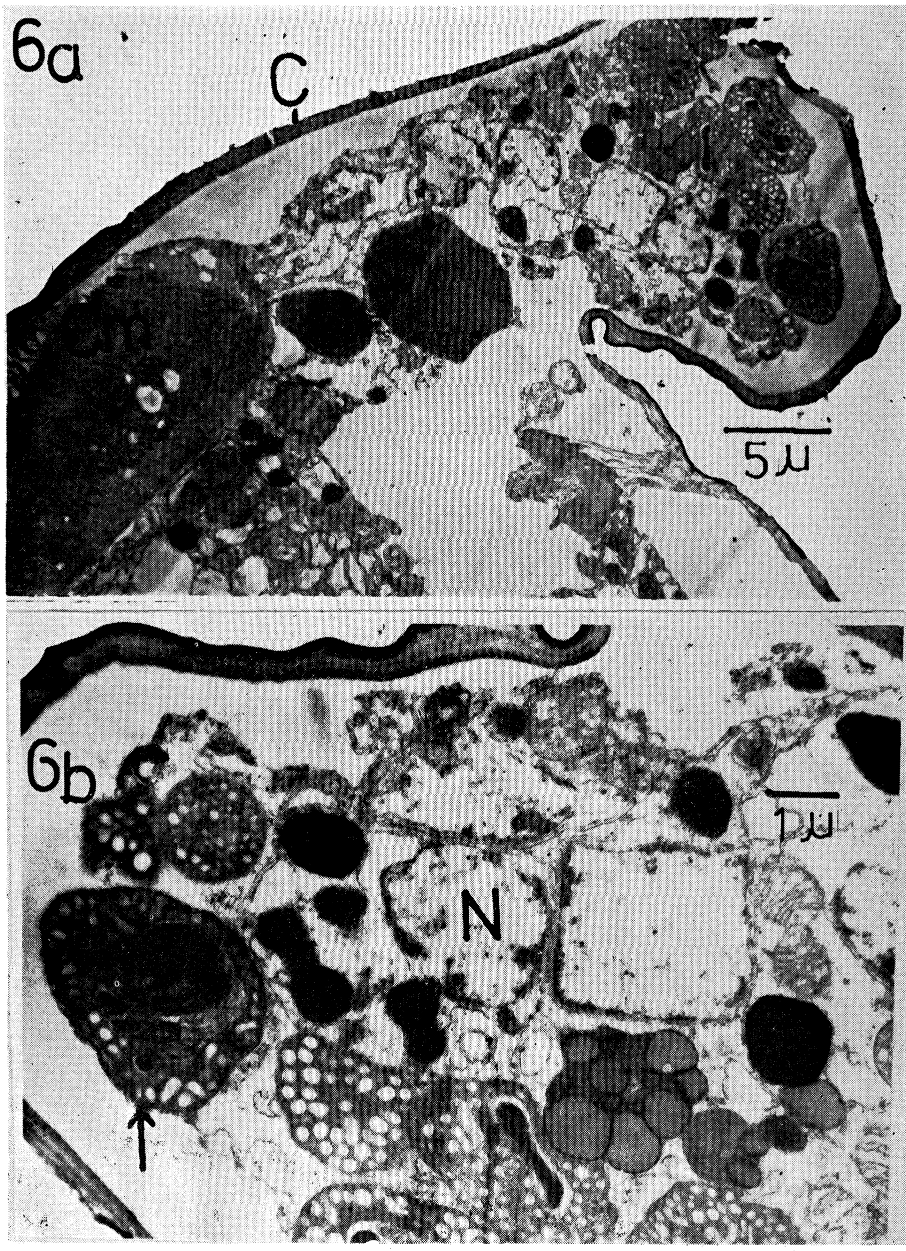

Fig. 6. Ultra-structure of male mites. a. Cross section of the male mite under the electron microscope, showing the caecum (Cm), cuticle (C), and other internal structures. b. Enlarged view of the right up side of (a) showing nucleus (N) and possible lysosome structures (Arrow) of the parenchyma cell.

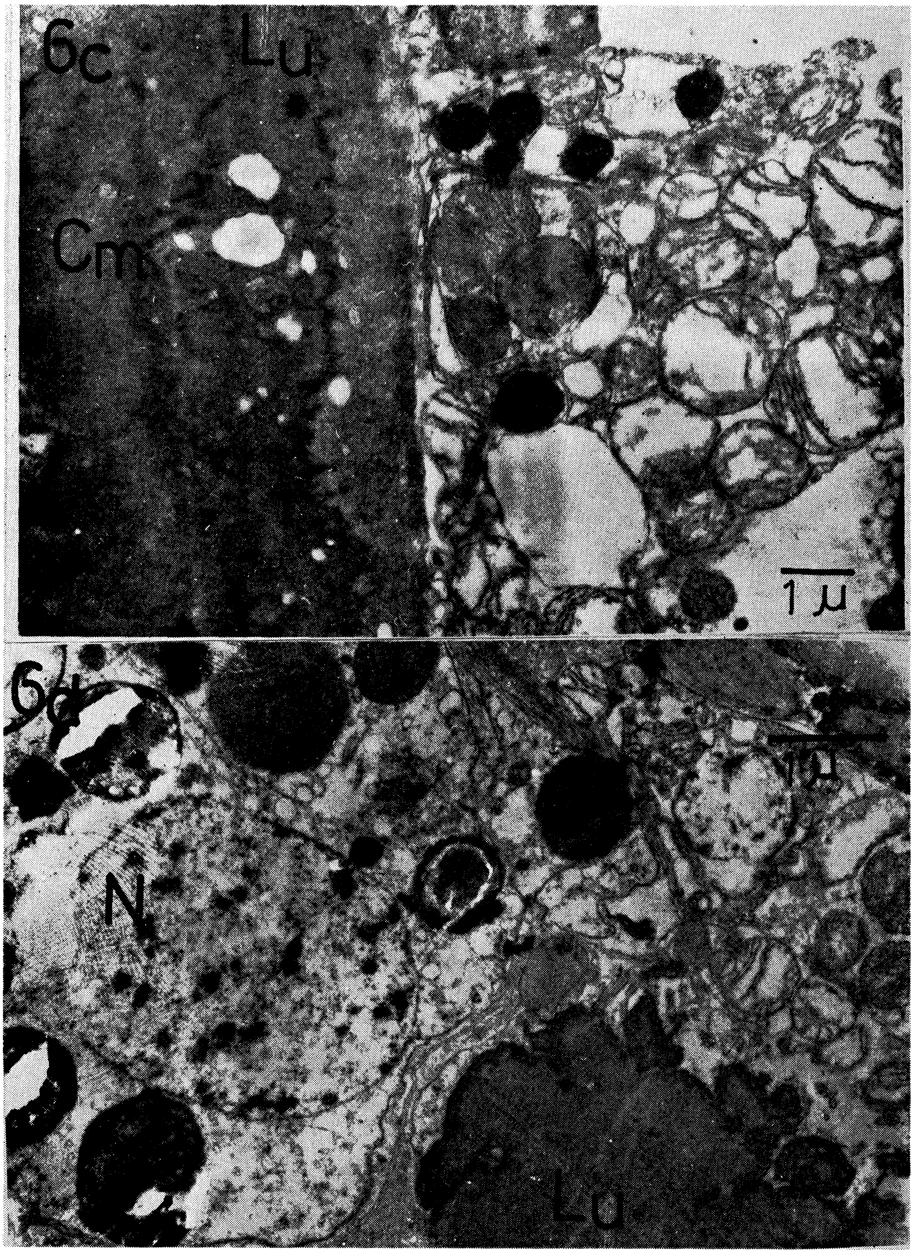

Fig. 6. c. Enlarged view of the longitudinal section of caecum showing the lumen (Lu) and many mitochondria to the left side. d. Cross section of the caecum cell nucleus (N) and lumen (Lu).

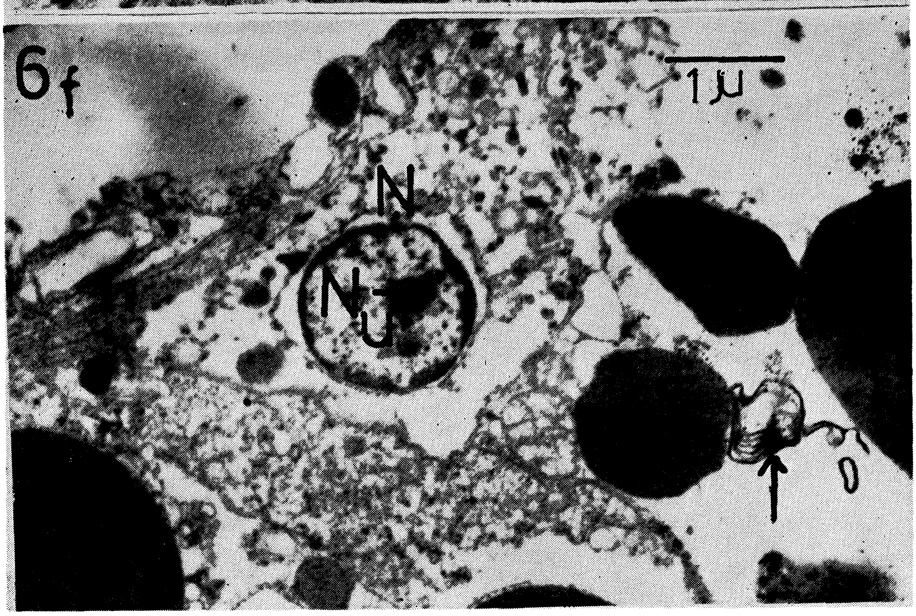

Fig. 6. e. Longitudinal section of the mite showing the epidermal cell organelles such as mitochondria, and muscle fibers. f. Enlarged view of the epidermal cell showing nucleolus (Nu) nucleus (N), and other myelin like body (arrow).

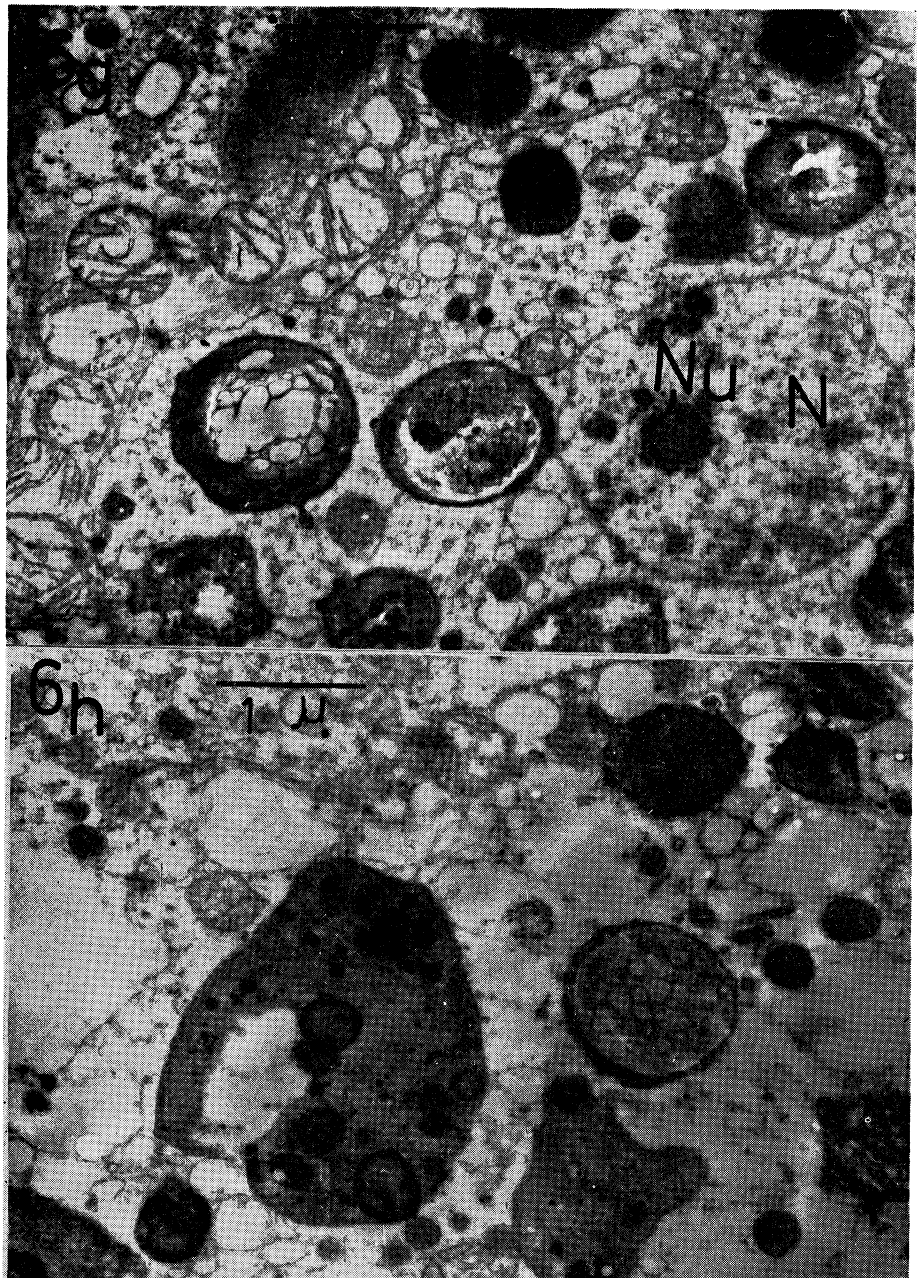

Fig. 6. g. Micrograph of mesenteron cell showing nucleus (N), nucleolus (Nu) and large inclusion body or granules. h. Enlarged view of (g) showing the details of inclusion bodys.

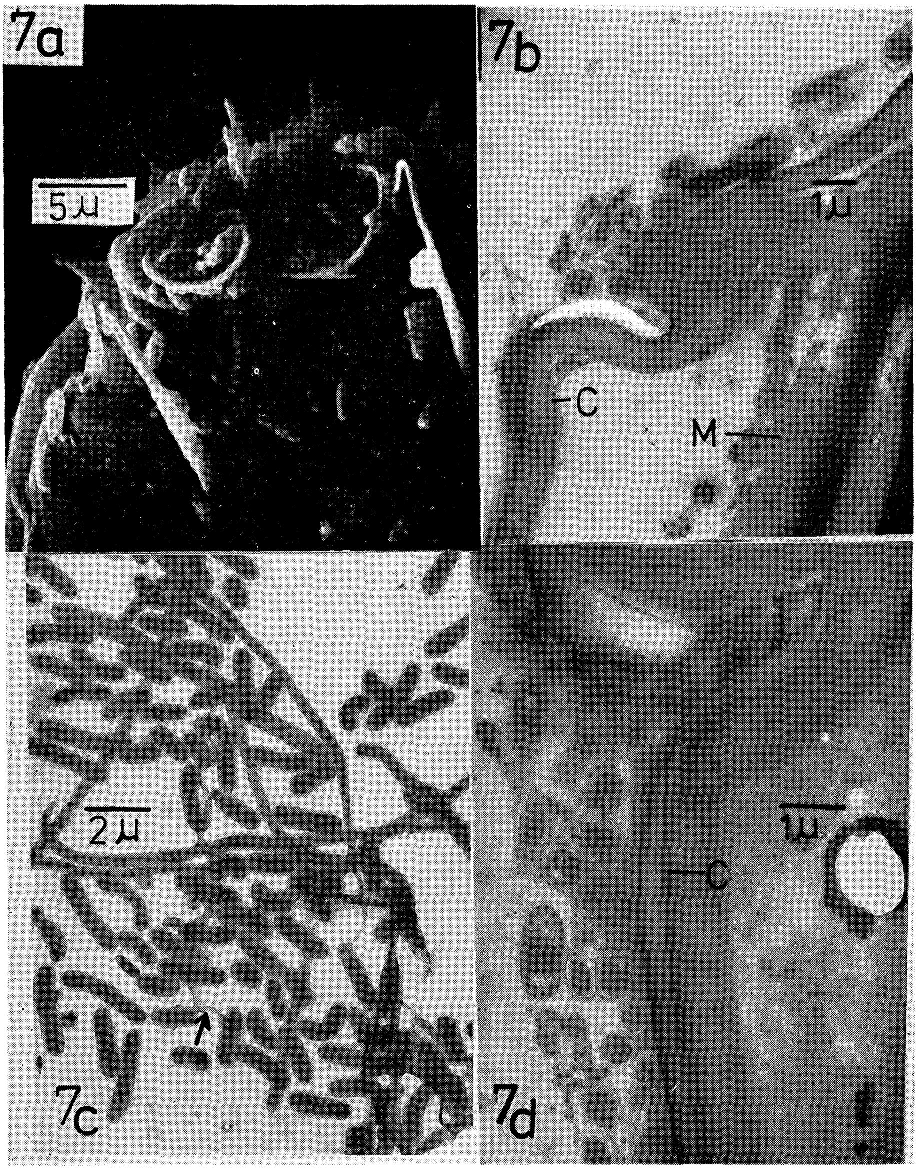

Fig. 7. The mite and the causal organism of rice sheath rot. a. Many conidia of *Acrocylsndrium oryzae* are attached to the outer surface of the mite and its mouthparts as viewed by scanning electron microscopy. b. Electron micrograph of the cross section of (a) showing the mite cuticle (C), muscle (M) and conidia (arrow) near the head region. c. Electron micrograph of a cultural line of the causal organism from the mite, its conidia and other mycelial materials (hyphae). Beside *A. oryzae*, other mycroplasma, like organism *Spiroplasma citri* (arrow) was occasionally found. d. Same as (b), but cross section near the mite body surface.